MEMORANDUM

DATE: July 19, 1999

TO: Antiviral Drugs Advisory Committee members

FROM: Rosemary Tiernan, M.D., Medical Officer
Cheryl Dixon, Ph.D., Statistician

VIA: Mark Goldberger, M.D., M.P.H., Director
Division of Special Pathogens and Immunologic Drug Products

SUBJECT: Clinical/Statistical Background Information for 27 July 1999 Advisory Committee Meeting

Drug name: Rapamune® (sirolimus) Oral Solution
NDA-21-083

Indication: Prevention of acute rejection in renal transplantation

Applicant: Wyeth-Ayerst Laboratories

I. Introduction:

This section of the FDA briefing package will focus on several specific safety issues that arose during the sirolimus Phase III trials i.e. pivotal studies 301 and 302. Comments regarding the design and conduct of the clinical studies will be rendered, and specific safety issues will be addressed in order to assist in a final risk/benefit analysis of the two proposed Rapamune doses.

Please refer to the Applicant's summary of safety data as presented in their Advisory Committee briefing package. Please also refer to the FDA Statistical Review which addresses the overall safety and efficacy of sirolimus oral solution, when used in conjunction with cyclosporine (CsA) and corticosteroids, to prevent acute rejection in the renal transplant recipient.

The recommended fixed maintenance dose for sirolimus is 2 mg/day (SRL 2). In addition, the Applicant is recommending a fixed maintenance dose of 5 mg/day (SRL 5) for patients with a "high risk for acute rejection" such as African-American patients, highly HLA mismatched patients, those with high panel reactive antibodies (PRA) and patients with a second transplant.

II. Design of Clinical Studies

The Applicant's briefing package summarizes the differences between pivotal studies 301 and 302. It is important to note that both studies were randomized, double-blind, controlled trials. The U.S. trial, study 301 (719 patients), utilized azathioprine as an

active control. Study 302 (576 patients), which included sites in the U.S., Europe and Canada, utilized a placebo control. The objective endpoints for both study 301 and 302 included a composite endpoint of acute rejection, graft loss (defined as nephrectomy or dialysis for 56 or more consecutive days) or death at 6 months and patient and graft survival at 12 months.

Reviewer's Note: ***A strength of study 301 was its enrollment of adequate numbers of African-American patients. The African-American segment of the U.S. transplant population is 21.3% according to 1998 UNOS data. In study 301, African-Americans comprised 23.1% of the study population.***

One potential weakness of U.S. study 301 was that, although the randomization at 48 hours post-transplant may have eliminated patients who sustained surgical site complications, it also may have eliminated "higher risk" patients such as those with "delayed graft function" (maintained on dialysis for at least one week post-transplant). However, study 302 randomized patients prior to transplantation and thus should capture those with delayed graft function.

Additional important points regarding studies 301 and 302:

Trial Design

1) Antibody induction was prohibited in study 301 and 302.
2) Antibody therapy (OKT3 or Anti-thymocyte globulin) was used for episodes of acute rejection, if the patient had failed an initial steroid "pulse". No Cellcept or FK506 was allowed.
3) Randomization was stratified by race, Black versus non-Black in study 301, and by donor origin, cadaver versus living donor, in study 302.
4) Study drug was administered as a loading dose of 15 mg and a fixed dose of 5 mg/day or as a loading dose of 6 mg with a fixed daily dose of 2 mg/day. Refer to section 7.10.8 through 7.10.8.3 of the Applicant's Advisory briefing package for a complete discussion of the issues related to drug exposure, dose reduction and discontinuations for sirolimus.
5) In the initial NDA submission, fourteen patients had been "lost-to follow-up" in study 301. However, over the past 6 months, the Applicant was able to obtain follow-up data on all patients except 5 in study 301: one in the SRL 2 mg arm , two in the SRL 5 mg arm and one in the azathioprine arm. If patients were lost to follow-up, they were counted as efficacy failures. No patients were lost to follow-up in study 302.
6) The Division of Scientific Investigations inspected three U.S. study sites and one overseas study site and found several minor problems with the conduct of studies 301 and 302. No major protocol deviations or violations were identified that would preclude accepting data from any individual study site. However, it should be noted that one study 301 investigator had a high rate of discontinuations at his site because he felt that when the patient's renal function began to worsen, it was imperative to discontinue study drug and initiate an alternative immunosuppressive regimen. There were also some difficulties in adhering to the time of randomization in study 302. Consequently, 67 of the 576 (12%) study 302 patients were assigned to treatment at one or more days after transplantation—as opposed to being randomized prior to transplantation. The Applicant

states that an analysis of the primary endpoint, after these patients were excluded, did not show a difference in the overall results. Consequently, it is doubtful that this will bias the study results.

7) Concomitant dosing for cyclosporine and corticosteroids was rendered as outlined in the Applicant's briefing package. The target cyclosporine troughs were slightly different for the first month in studies 301 and 302.

Target **cyclosporine** troughs were:

	Study 301	**Study 302**
Month 1	200-350 ng/ml	200-400 ng/ml
Month 2-3	200-300 ng/ml	200-300 ng/ml
≥Month 3	150-250 ng/ml	150-250 ng/ml

Corticosteroids were dosed as :
pulse corticosteroids on days 1-4
tapering to 10mg/day by month 6
between 5-10 mg/day by month 12

Azathioprine, the active comparator in study 301, was dosed at 2-3mg/kg/day.

8) Prophylaxis for *Pneumocystis carinii* pneumonia was mandated for one year post-transplant during this trial. CMV prophylaxis for "high risk" patients, i.e. CMV negative recipient of CMV donor positive kidney, was mandated for 3 months post transplant (using center-specific therapy such as ganciclovir or acyclovir) and was also recommended for lower risk renal transplant recipients. Prophylaxis for urinary tract infections was routinely utilized for 6 weeks post-transplant and the study centers chose the antibiotic according to their standard practice.

Demographics

1) Multi-organ transplants and high risk patients, as defined by the criteria of high PRA or second transplant were excluded from studies 301 and 302.

1) Study 301 had 35.2% living related (LRT) and living unrelated donors (LURT). Study 302 had 23% LRT and LURT.

3) Studies 301 and 302 enrolled different ethnic populations and thus there may be differences in dietary habits and reporting of adverse events.

4) A significantly higher proportion of females was assigned to the azathioprine arm in study 301.

5) The majority of renal allograft donors were of white ethnic background and were CMV positive.

6) Glomerulonephritis and hypertension were the most common etiologies for end stage renal disease in renal transplant recipients.

III. EFFICACY ISSUES

Please refer to the FDA statistical review on efficacy for further discussion regarding the results summarized below.

The primary objectives of studies 301 and 302 were to evaluate superiority of sirolimus compared to azathioprine (study 301) and placebo (study 302) with respect to efficacy failure and to exclude that patient and graft survival were unacceptably impaired, based on the 97.5% confidence intervals of the differences in survival rates. Thus, the primary efficacy endpoints were efficacy failure at 6 months, patient and graft survival at 12 months. Efficacy failure was defined as biopsy proven acute rejection, graft loss or death. Patients lost to follow-up at 6 months were treated as efficacy failures in the primary analysis.

In the primary, intent-to- treat, analysis of study 301 the overall rate of efficacy failures were 18.7% (53/284) for sirolimus 2mg/day, 16.8% (46/274) for sirolimus 5mg/day and 32.3% (52/161) for azathioprine.

In the primary, intent-to- treat, analysis of study 302 the overall rate of efficacy failures were 30.0% (68/227) for sirolimus 2mg/day, 25.6% (56/219) for sirolimus 5mg/day and 47.7% (62/130).

Reviewer's Note: ***Sirolimus 2mg/day and 5mg/day significantly reduced the incidence of efficacy failure compared to azathioprine or placebo during the first six months post transplantation.***

Randomization in study 301 was stratified by race, Black versus non-Black. Among Blacks the rates of efficacy failure were 34.9% (22/63) for sirolimus 2mg/day, 18.0% (11/61) for sirolimus 5 mg/day and 33.3% (14/42) for azathioprine.

Reviewer's Note: ***Efficacy failure was slightly higher at 2mg/day and not statistically significantly superior at 5 mg/per day compared to azathoprine in this population. Pharmacokinetic analyses did not demonstrate that Back patients had lower levels of either cyclosporine or siroloimus. Thus, the differences in outcome by race cannot be explained by different exposures to these drugs.***

Additional studies are needed to ascertain the factors that contribute to the higher rate of efficacy failure in the black patients who received sirolimus 2 mg/day. The Applicant is recommending that the higher dose of sirolimus 5 mg/day be used in black patients as well as other "high risk" groups. However, the factors that cause this reduced efficacy in black patients do not appear to be related to pharmacodynamic issues. Consequently, any increase in sirolimus or cyclosporine dose must be weighed against the side-effects of these drugs, including but not limited to the problems associated with "over-immunosuppression" and dose-related adverse events.

Tables 1 and 2 summarize efficacy failure at 6 months in selected subgroups of particular interest in studies 301 and 302 respectively.

Table 1
Efficacy Failure at 6 months
Selected subgroups in Study 301

Subgroup	SRL 2 mg/day (n=284)	SRL 5 mg/day (n=274)	Azathioprine (n=161)
Recipient Race			
Blacks	22/63 (34.9)	11/61 (18.0)	14/42 (33.3)
Non-blacks	31/221 (14.0)[c]	35/213 (16.4)[c]	36/119 (30.3)
Recipient Gender			
Female	14/76 (18.4)	20/103 (19.4)	17/71 (23.9)
Male	39 /208 (18.8)[c]	26/171 (15.2)[c]	35/90 (38.9)
Donor Source			
Cadaver	39/180 (21.7)	28/167 (16.8)[a]	34/119 (28.6)
Living Related	10/86 (11.6)[c]	15/83 (18.1)[b]	14/33 (42.4)
Living Unrelated	4/18 (22.2)	3/24 (12.5)	4/9 (44.4)
Number of HLA mismatches			
0 to 2	12/69 (17.4)	8/69 (11.6)	7/42 (16.7)
3 to 6	41/215 (19.1)[c]	38/205 (18.5)[c]	45/119 (37.8)

a: Comparison with azathioprine statistically significant at less than 0.05.
b: Comparison with azathioprine statistically significant at less than 0.01
c: Comparison with azathioprine statistically significant at less than 0.001.

Table 2
Efficacy Failure at 6 months
Selected subgroups in Study 302

Subgroup	SRL 2 mg/day (n=227)	SRL 5 mg/day (n=219)	Placebo (n=130)
Recipient Race			
Blacks	8/26 (30.8)	9/27 (33.3)	5/13 (38.5)
Non-blacks	60/201 (29.9)[c]	47/192 (24.5)[c]	57/117 (48.7)
Recipient Gender			
Female	27/79 (34.2)	21/70 (30.0)	16/39 (41.0)
Male	41/148 (27.7)[c]	35/149 (23.5)[c]	46/91 (50.6)
Donor Source			
Cadaver	54/173 (31.2)[a]	48/174 (27.6)[b]	43/99 (43.4)
Living Related	14/39 (35.9)	5/29 (17.2)[b]	16/27 (59.3)
Living Unrelated	0/15 (0.0)[b]	3/16 (18.8)	3/4 (75.0)
Number of HLA mismatches			
0 to 2	13/51 (25.5)	10/60 (16.7)	7/30 (23.3)
3 to 6	55/176 (31.3)[c]	46/159 (28.9)[c]	55/100 (55.0)

a: Comparison with azathioprine statistically significant at less than 0.05.
b: Comparison with azathioprine statistically significant at less than 0.01
c: Comparison with azathioprine statistically significant at less than 0.001.

Reviewer's Note: Among women there were no statistically significant differences in efficacy failure across treatment groups.

In analyses of data from registries of renal transplantation, recipients of cadaver organs are at greater risk for rejection and graft loss compared to recipients of organs from living donors. Though not statistically significant, a larger proportion of subjects

received organs from living donors in the sirolimus treatment groups compared to the azathioprine control group in study 301. Rates of efficacy failure among living donor organ recipients were significantly decreased in sirolimus treatment groups compared to azathioprine. Rates of efficacy failure for recipients of cadaver organs assigned to treatment with sirolimus 2mg per day were significantly decreased compared to azathioprine and only marginally decreased at 5 mg per day. Rates of efficacy failure among cadaver organ recipients were not significantly improved for sirolimus 5mg per day compared to 2 mg per day in study 301 and 302.

The quality of HLA match between donors and potential recipients of kidney allografts is an important criteria in the UNOS organ allocation system and a predictor of graft survival. Overall, rates of efficacy failure were greater among patients with 3 to 6 HLA mismatches compared to those with 0 to 2 HLA mismatches. Although the former were a greater risk for efficacy failure they did not appear to significantly benefit from sirolimus 5 mg per day compared to 2 mg per day.

Overall patient and graft survival in were at 12 months in study 301 were 94.7% (269/284) for sirolimus 2mg per day, 92.7% (254/274) for sirolimus 5 mg per day, and 93.8% (151/161) for azathioprime by intent-to-treat analysis. The 97.5% confidence intervals for the differences in patient and graft survival at 12 months (rate for sirolimus minus rate for azathioprine control) were –4.8% to +6.6% for sirolimus 2 mg per day, and –7.1% to +4.9 % for sirolimus 5 mg per day.

Overall patient and graft survival in were at 12 months in study 302 were 89.9% (204/227) for sirolimus 2mg per day, 90.9% (190/219) for sirolimus 5 mg per day, and 87.7% (114/130) for placebo by intent-to-treat analysis. The 97.5% confidence intervals for the differences in patient and graft survival at 12 months (rate for sirolimus minus rate for placebo control) were –6.3 to +10.7% for sirolimus 2 mg per day, and —5.2% to +11.6 % for sirolimus 5 mg per day.

<u>Reviewer's Note:</u> Patient and graft survival were excellent in both studies and the 97.5% confidence intervals for differences in survival included zero. The overall treatment effects observed on biopsy proven acute rejection were not associated with a detectable improvement in patient or graft survival at one year.

The greatest decrease in survival that one could exclude in study 301 with 97.5% confidence compared to the azathioprine control group, was –4.8% for sirolimus 2mg/per day and –7.1% for sirolimus 5 mg/per day. In study 302 the greatest decrease in survival that one could exclude with 97.5% confidence compared to placebo was – 6.3% for sirolimus 2 mg per day, and –5.2% for sirolimus 5 mg per day. In considering the efficacy of sirolimus, one must consider whether the risk of such decreases would be acceptable.

In studies 301 and 302, the use of anti-T-lymphocyte antibody therapies to treat the first biopsy-confirmed acute rejection during the first 6 months post-transplant was significantly reduced for sirolimus 5 mg compared to the control groups.

Reviewer's note: ***This decrement in the use of anti- T-lymphocyte antibody preparations was a secondary study endpoint and did not translate into improved survival, decreased rate of infection or decreased rate of post-transplant lymphoproliferative disease (PTLD). In fact, the incidence of PTLD was highest in the sirolimus 5 mg study arm.***

IV. Safety

Of the 1295 patients enrolled in studies 301 and 302, 1260 patients received randomized treatment and were considered evaluable for safety.

1. Please refer to the Applicant's briefing package for more detailed information regarding deaths and graft loss.

The most common reasons for deaths were vascular (cardiovascular or cerebrovascular) and infection. The overall death rate in study 301 and 302 was 3.7%. This included death rates of :

3.1% sirolimus 2 mg/day
4.5% sirolimus 5 mg/day
1.9% Azathioprine
5.4% Placebo

Table 3 Study 301Summary of Deaths, Graft Loss, Malignancy, Life-Threatening Adverse Events

Event	SRL 2 mg/day (n=284)	SRL 5 mg/day (n=274)	Aza (n=161)
Death	3 (1.1)	8 (2.9)	3 (1.9)
Graft Loss	4 (1.4)	12 (4.3)	7 (4.3)
Malignancy	1 (0.4)	2 (0.7)	3 (1.9)
Life-Threatening Adverse Event	6 (2.1)	4 (1.5)	0

Table 4 Study 302

Summary of Deaths, Graft Loss, Malignancy, Life-Threatening Adverse Events

Event	SRL 2 mg/day (n=227)	SRL 5 mg/day (n=219)	Placebo (n=130)
Death	5 (2)	8(4)	6 (5)
Graft Loss	16 (7)	16 (7)	15 (12)
Malignancy	3 (1)	2 (1)	1 (1)
Life-Threatening Adverse Event	1 (<1)	2 (1)	1 (1)

2. The most common etiology of graft loss was death with a functioning graft and the second most common etiology was acute rejection. Please refer to the Applicant's briefing package for a full discussion.

3. The incidence of malignancy (post-transplant lymphoproliferative disease PTLD/lymphoma) during the first year post-transplant was higher in the sirolimus 5 mg/day group with an overall incidence of 1.4%. The rates of PTLD in this trial were similar to that which has been reported in other trials of immunosuppressive agents.

Reviewer's note: ***Epstein-Barr virus (EBV) serologies were not collected on patients at study onset and thus I can not comment on whether the cases of PTLD were in "high risk" EBV-negative transplant recipients of EBV-positive donor kidneys.***

4. Please refer to the Applicant's briefing package regarding a discussion on discontinuation of study drug. The most frequent reason for discontinuation in the sirolimus 2 mg/day group was an unsatisfactory response and in the sirolimus 5 mg group it was an adverse event. Tables 5 and 6 below (and Tables 18 and 21 of the FDA statistical review) outline the clinically important treatment emergent adverse events (TEAE) that occur in studies 301 and 302 respectively. These tables also demonstrate the side effects that appear to be dose-dependent. Hypertension, diarrhea, anemia, leukopenia, thrombocytopenia and hyperlipidemia show an increased frequency at the higher dose of sirolimus.

Table 5

Number (%) of **Study 301** Patients Reporting Clinically Important TEAE Excluding Infection and Malignancy

Body system Event	**SRL 2 mg/day** (n=281)	**SRL 5 mg/day** (n=269)	**Azathio-prine** (n=159)	p-value
Body as a whole				
Headache	44 (16)	50 (19)	12 (8)	0.005*
Lymphocele	33 (12)	36 (13)	4 (3)	<0.001*
Cardiovascular system				
Hypertension	96 (34)	89 (33)	35 (22)	0.017*
Digestive system				
Diarrhea	50 (18)	74 (28)	18 (11)	<0.001*
Liver function tests abnormal	24 (9)	26 (10)	14 (9)	0.381
Endocrine system				
Diabetes mellitus	14 (5)	22 (8)	8(5)	0.256
Hemic and lymphatic system				
Anemia	56 (20)	73 (27)	32 (20)	0.096
Leukopenia	14 (5)	28 (10)	17 (11)	0.027*
Thrombocytopenia	25 (9)	47 (17)	9 (6)	<0.001*
Thrombotic thrombocytopenia purpura (TTP)	2 (<1)	4 (1)	0	0.283
Metabolic and nutritional				
Creatinine increased	61 (22)	64 (24)	32 (20)	0.669
Healing abnormal	22 (8)	24 (9)	6 (4)	0.120
Hypercholesteremia	84 (30)	94 (35)	34 (21)	0.012*
Hyperglycemia	34 (12)	39 (14)	18 (11)	0.581
Hyperkalemia	34 (12)	22 (8)	30(19)	0.005*
Hyperlipemia	83 (30)	103 (38)	29 (18)	<0.001*
Peripheral edema	137 (49)	134 (50)	68 (43)	0.343
Musculoskeletal system				
Arthralgia	40 (14)	53 (20)	18 (11)	0.053
Nervous system				
Hypotonia	14 (5)	14 (5)	1 (<1)	0.022*
Insomnia	28(10)	51 (19)	19 (12)	0.008*
Tremor	60 (21)	64 (24)	25 (16)	0.136
Respiratory system				
Epistaxis	8 (3)	13 (5)	1 (<1)	0.043*
Skin and appendages				
Acne	67 (24)	49 (18)	18 (11)	0.004*
Hirsutism	14 (5)	32 (12)	3 (2)	<0.001*
Rash	23 (8)	19 (7)	3 (2)	0.016*

*Overall difference among treatment groups assessed by Fisher's exact test.

Reviewer's note: ***Some adverse events commonly associated with cyclosporine such as hypertension, tremor, headache and hirsutism were more frequently reported in the sirolimus treatment groups in study 301.***

Table 6
Number (%) of **Study 302** Pts. Reporting Clinically Important TEAE Excluding Infection and Malignancy

Body system Event	SRL 2 mg/day (n=218	SRL 5 mg/day (n=208)	Placebo (n=124)	p-value
Body as a whole				
Headache	55 (25)	57 (27)	23 (19)	0.184
Lymphocele	20 (9)	25 (12)	6 (5)	0.091
Cardiovascular system				
Hypertension	80 (37)	84 (40)	51 (41)	0.611
Digestive system				
Diarrhea	36 (16)	50 (24)	17 (14)	0.038*
Endocrine system				
Diabetes mellitus	9 (4)	16 (8)	3 (2)	0.082
Hemic and lymphatic system				
Anemia	36 (16)	56 (27)	16 (13)	0.003*
Leukopenia	15 (7)	18 (9)	3 (2)	0.069
Thrombocytopenia	25 (11)	47 (23)	4 (3)	<0.001*
Thrombic thrombocytopenia purpura (TTP)[1]	4 (2)	7 (3)	2 (2)	0.586
Metabolic and nutritional				
ALT increased	19 (9)	20 (10)	9 (7)	0.777
AST increased	9 (4)	15 (7)	6 (5)	0.369
Creatinine increased	56 (26)	65 (31)	40 (32)	0.295
Healing abnormal	15 (7)	22 (11)	7 (6)	0.224
Hypercholesteremia	81 (37)	91 (44)	25 (20)	<0.001*
Hyperglycemia	23 (11)	25 (12)	12 (10)	0.786
Hyperkalemia	29 (13)	23 (11)	28 (23)	0.016*
Hyperlipemia	76 (35)	103 (50)	22 (18)	<0.001*
Peripheral edema	93 (42)	98 (47)	43 (35)	0.086

*Overall difference among treatment groups assessed by Fisher's exact test.
[1]All patiens with hemolytic uremic syndrome (HUS) were coded to this term.

5. The major toxicities that I will now focus on will include: infection, hyperlipidemia, post-transplant diabetes mellitus, elevated liver function tests , hematologic toxicity, HUS/TTP and renal function.

Reviewer's note: ***The applicant recommends that "high risk patients" be administered the sirolimus 5 mg/day dose. They infer that the African-American population incurred less side effects/less risk from sirolimus. Please keep in mind that there were only 166 black patients in study 301. Regarding complications from cytomegalovirus infection—the African-American patients may have been a lower risk population to develop serious CMV infection and serious CMV disease.***

A. INFECTION

1) There was a decreased incidence of CMV in studies 301 and 302 that the Applicant partially attributed to the use of CMV prophylaxis. However, assessment of the

degree of CMV donor and recipient mismatch for study 301 demonstrated that the majority of the black and non-black patients in study 301 were not at high risk to develop serious CMV infection or disease i.e. they were not CMV negative recipients (R-) of CMV positive donor kidneys (D+) i.e. (CMV D+R-).

Reviewer's note: The applicant recommends that "high risk patients" be administered the sirolimus 5 mg/day dose. They claim that the black population incurred less side effects/less risk from sirolimus. Please keep in mind that there were only 166 African-American patients in study 301. Regarding complications from cytomegalovirus infection—the African-American patients may have been a low risk population to develop serious CMV infection and serious CMV disease.

Table 7 Analysis Black patients with "high risk" to develop serious CMV infection and disease (CMV D+R-)

Study 301 Treatment Arms	Black Pts. in 301	Black patients CMV D+/R- "high risk"	Black Pts. with unknown D/R serologic status for CMV
AZA	42	5/42 (11.9%)	4
SRL 2	63	4/63 (6.3%)	4
SRL 5	61	2/61 (3.3%)	3
Total Black patients	166	11/166 (6.6%)	11 (6.6%)

Table 8 Analysis Non-Black patients with "high risk" to develop serious CMV infection and disease (CMV D+R-)

Study 301 Treatment Arms	Non-Black Pts. in 301	Non-Black patients CMV D+/R- "high risk"	Non-Black with unknown serologic status for CMV
AZA	119	21/119 (17.6%)	0
SRL 2	221	55 /221 (25.0%)	3
SRL 5	213	46 /213 (21.6%)	5
Total Non-Black patients	553	122/553 (22.1%)	8 (1.5%)

***Reviewer's note:* The Applicant has made the argument that there were less opportunistic infections in the African-American population in study 301 and thus they could better tolerate increased immunosuppression. However, as one can see in the above tables, the percentage of black patients in study 301, who were at high risk to develop CMV infection and disease, was only 6.6%. The non-black patients in study 301 carried a "high risk" of 22.1%. Study 302 enrolled less black patients. However,**

once again, of the 66 black patients enrolled in study302, only 2 were CMV negative recipients of CMV positive donor kidneys. Many different host and epidemiologic factors, as well as the level of immunosuppression, contribute to the development of post-transplant infection. Unless one is able to account for all of these factors, and in light of the fact that by chance the African-American population in this study may have been at a lesser risk for serious CMV infection, it may be premature to conclude that this population would better tolerate increased doses of sirolimus or cyclosporine.

2) There was no increase in the rates of sepsis, pyelonephritis, wound infection and pneumonia across treatment groups in studies 301 and 302.

3) There was no increase in the incidence of opportunistic infection in either of the sirolimus treatment groups compared to the control groups in studies 301 and 302, except for a higher incidence of mucosal *Herpes simplex* in the sirolimus 5 mg group.

4) Despite differences between treatment groups, with respect to episodes of acute rejection requiring additional high doses of immunosuppression, there were no significant differences between treatment groups with respect to serious infection.

Reviewer's note: ***The increased incidence of mucosal herpes simplex is quite unusual considering many of these patients were receiving either acyclovir or ganciclovir prophylaxis for CMV infection. Either of these two antiviral drugs has efficacy against Herpes simplex virus. Please note that the diagnosis of Herpes simplex infection can be problematic in that it was not confirmed by laboratory tests such as culture.***

B) HYPERLIPIDEMIA

Reviewer's note: ***The following tables pertain to an analysis of treatment emergent abnormalities in serum cholesterol and triglycerides that developed in transplant recipients in Studies 301 and 302 .Data was not collected for HDL, LDL or apolipoproteins during Studies 301 and 302. Consequently, the following analysis utilizes a threshold for "normal cholesterol" as < 200 mg/dl and "elevated cholesterol" as ≥240 mg/dl. Keep in mind that the National Cholesterol Education Program (NCEP) guidelines for intervention utilizing lipid-lowering agents relies on data that was not available for our review such as LDL values and cardiac risk factors. The threshold values utilized for the triglyceride analysis include a "normal triglyceride" value of <200 mg/dl and "elevated triglyceride" value of ≥500 mg/dl.***

The lipid analysis below differs from the Applicant's analysis in that it evaluates a cohort of patients who had normal cholesterol and triglyceride levels prior to initiation of study drug and who developed hyperlipidemia while on study drug. Hyperlipidemia has been identified as a major side-effect with sirolimus and has surfaced in all Phase II and Phase III studies.

TABLE 9 Study 301 patients who developed hypercholesterolemia on study drug

# study 301 patients	Azathioprine	Sirolimus 2mg	Sirolimus 5mg
Total # study patients in each treatment arm	161	284	274
Pts. with pre-study chol.<200mg/dl	116/161 (72.1%)	204/284 (71.8%)	195/274 (71.2%)
Pts. with normal baseline cholesterol who developed chol. ≥240 mg/dl on study drug	55/116 (47.4%)	131/204 (64.2%)	133/195 (68.2%)
Fisher's exact p-value		0.005	0.0003

TABLE 10 Study 302 Patients who developed hypercholesterolemia on study drug

# study 302 patients	Placebo	Sirolimus 2 mg	Sirolimus 5 mg
Total # patients in each treatment arm	130	227	219
Pts. with pre-study chol.<200mg/dl	95/130 (73.1%)	163/227 (71.8%)	165/219 (75.3%)
Pts. with normal baseline cholesterol who developed chol. ≥240 mg/dl on study drug	39/95 (41.1%)	123/163 (75.5%)	120/165 (72.7%)
Fisher's exact p-value		<0.0001	<0.0001

Reviewer's note: ***A significant risk to develop new onset hypercholesterolemia , above and beyond the risk anticipated from cyclosporine, exists in the sirolimus treatment arm and was identified in both study 301 and 302.***

TABLE 11 Study 301 patients who developed hypertriglyceridemia on study drug

Study 301 patients	AZA	Sirolimus 2 mg	Sirolimus 5 mg
Total # patients in each treatment arm	161	284	274
Pts. with pre-study TG≤200mg/dl	121/161 (75.2%)	207/284 (72.9%)	229/274 (83.6%)
Pts. with normal baseline TG who developed TG >500 mg/dl on study drug	6/121 (5.0%)	30/207 (14.5%)	41/229(17.9%)
Fisher's exact p-value		0.01	0.0005

TABLE 12 Study 302 patients who developed hypertriglyceridemia on study drug

Study 302 patients	Placebo	Sirolimus 2 mg	Sirolimus 5 mg
Total # patients in each treatment arm	130	227	219
Pts. with pre-study TG≤200mg/dl	89 (68.5%)	168 (74.0%)	170 (77.6%)
Pts. with normal baseline TG who developed TG >500 mg/dl on study drug	2 (2.2)	26 (15.5)	40 (23.5)
Fisher's exact p-value		0.0006	<0.0001

Reviewer's note: A significant risk to develop new onset hypertriglyceridemia, above and beyond the risk anticipated from cyclosporine, exists in the sirolimus treatment arms and has been identified in both study 301 and 302.

Table 13 Analysis of the use of lipid lowering agents Study 301

Study 301	AZA	SRL 2 mg	SRL 5 mg
Patients with normal cholesterol pre-study	116	204	195
Patients initiated on lipid -lowering drug	25(21.6%)	93 (45.6%)	101 (51.8%)
Patients who continued on lipid lowering drug at 6-12 months	23(20%)	59 (29%)	69 (35%)

Table 14 Analysis of the use of lipid lowering agents Study 302

Study 302	Placebo	SRL 2 mg	SRL 5 mg
Patients with normal cholesterol pre-study	95	163	165
Patients initiated on lipid -lowering drug	15 (15.8%)	69 (42.3%)	77 (46.7%)
Patients who continued on lipid lowering drug at 6-12 months	11 (12%)	47 (29%)	64 (39%)

Reviewer's note: In study 301, approximately 22% of the patients with normal cholesterol at study onset who developed hypercholesterolemia on azathioprine as did 46 to 52 %. In study 302, approximately 16% of the azathioprine patients were initiated on lipid-lowering agents and 42-47% of the patients on sirolimus. Once initiated on a lipid lowering agent, at least 60 % of these patients continued on the lipid lowering agent at study's end. The majority of the lipid- lowering agents used were HMG-CoA reductase inhibitors.

Reviewer's comment: As seen in the above tables, it is obvious that a significant proportion of patients who entered these trials with normal lipid profiles, and were treated with sirolimus, developed a new problem with either elevated cholesterol and/or elevated triglycerides. The Applicant states that this problem was manageable with diet, exercise, lipid -lowering agents, reduction in corticosteroids and cyclosporine and that there was no evidence of major vascular disease at the end of one year. However, one year is too early to assess the major sequelae of this hyperlipidemia. Please also keep in mind that these patients may carry additional risk factors for heart disease such as family history, diabetes and hypertension. Values for HDL, LDL and the apolipoproteins were not collected during this trial and consequently it was not possible to include these parameters in the assessment of hyperlipidemia. We looked at the potential role of elevated cyclosporine/sirolimus levels contributing to hyperlipidemia, but found no data to substantiate a correlation. There was no significant increase in hyperlipidemia, in this group of patients with normal baseline lipid values, when the higher sirolimus dose was utilized. The demographics showed that non-Black male patients tended more often to develop hypercholesterolemia on sirolimus 2 mg and 5 mg. Non-Black females developed more problems with hypercholesterolemia on azathioprine. If a patient with a normal pre-study cholesterol developed hypercholesterolemia on any study drug and was initiated on lipid lowering therapy, greater than 60% of those patients continued to require the lipid lowering agent at 6-12 months post- transplant.

C. Post-transplant diabetes mellitus (PTDM)

PTDM was defined as a patient, without a prior history of IDDM or NIDDM, and who requires the use of insulin for 30 or more consecutive days with less than 5 days of interruption to maintain a normal, fasting blood glucose concentration.
Twenty seven patients fit the above criteria.

TABLE 16. 27 Patients who developed PTDM on study 301 & 302

	Placebo N= 84	Azathioprine N= 98 (23 Black 75 Non-Black)	Sirolimus 2 mg N= 334 (51 Black 283 Non-black)	Sirolimus 5 mg N= 327 (52 Black 275 Non-black)
Male	0	2	9	7
Female	0	0	1	8
Black	0	2	3	6
Non-Black	0	0	7	9
Total # pts.	0	2	10	15
Incidence PTDM	0%	2%	3%	4.6%

Reviewer's note: Overall, the incidence of PTDM was uncommon in study 301 and 302. However, despite greater rates of acute rejection and use of additional steroids to treat the episodes of rejection in the control arms, there was no corresponding increase in PTDM. In fact, the incidence of PTDM is greater in the sirolimus 5 mg study arm, noting that there were 8 patients whose status regarding the use of insulin was unclear (2 in the azathioprine arm and 6 in the sirolimus 5 arm). African-Americans did not appear to be at increased risk to develop PTDM in this study.

D. Liver function Tests (LFT's)

Reviewer's note: Please note that information regarding the serologic status of study patients for Hepatitis B or C was not reported in this study. The LFT's that were assessed included alkaline phosphatase, AST,and ALT . Bilirubin levels were not collected. Please refer to table 8.71a in the Applicant's briefing package. Essentially the percentage of patients who developed elevations of these LFT parameters to 5 and 10 times the upper limit of normal were equally distributed among the study drug groups in studies 301 and 302 . The overall percentage of LFT elevations was small and no significant trends were identified by race or gender.

E. Renal Function as measured by Nankivell GFR and serum creatinine

Reviewer's comment : Sirolimus is believed to lack inherent nephrotoxicity as data indicates in animal models and in the phase 2 trials in de novo renal transplants and in phase 2 monotherapy psoriasis trials. In the following tables, one can see that in black and non-black patients the GFR was better in the group on AZA at 12 months. In the briefing package, the Applicant notes that patients on CsA and sirolimus have higher creatinine levels over time vs patients treated with full dose CsA in conjunction with placebo or azathioprine. These creatinine levels show a dose relationship with higher levels of creatinine found in patients treated with SRL 5 mg. The Applicant claims that this is mainly due to CsA nephrotoxicity. Our analysis is different than that of the

Applicant's which was an "on treatment" analysis. Our analysis attempted to include all patients who had a value for creatinine and/or GFR at 12 months whether or not they were currently on study drug. This population remained similar to the overall study populations in study 301 and 302 with respect to rates of acute rejection, and time to rejection.

Table 17 Study 301 GFR Results at 12months (337-393 days)

Treatment	N (observed) /N (total)	Mean GFR (cc/min) +/- SD	p-value
Azathioprine	127/161 (78.9%)	65.9 +/- 19	----------
SRL 2 mg	233/284 (82.0%)	57.4 +/- 19.6	0.001
SRL 5 mg	226/274 (82.5%)	55.1 +/- 19.3	0.001

Tabie 18 Study 301 Creatinine at 12 months (337-393 days)

Treatment	N(observed)	Mean creatinine mg/dl +/- SD	p-value
Azathioprine	127	1.6 +/- 0.63	
Sirolimus 2	233	2.17 +/- 1.49	0.0001
Sirolimus 5	227	2.09 +/- 1.36	0.0002

***Reviewer's note:** In study 301 both GFR and serum creatinine are significantly better in the azathioprine arm at 12 months. The serum creatinine is better for both blacks and non-blacks in the azathioprine arm at 12 months. There is no statistical improvement in the serum creatinine with sirolimus 5 in the African-American population at 12 months.*

Table 19 Study 302 GFR results at 12 months (337-393 days)

Treatment	N observed /N total	Mean GFR (cc/min) +/- SD	p-value
Placebo	101/130 (77.7%)	61.7 +/- 18.18	-----
SRL 2 mg	190/227 (83.7%)	54.9 +/- 17.36	0.0022
SRL 5 mg	175/219 (79.9%)	52.9 +/- 18.29	0.001

Table 20 Study 302 Creatinine at 12 months

Treatment	N observed	Mean creatinine mg/dl +/- SD	p-value
placebo	102	1.96 +/- 1.77	
SRL 2	191	2.11 +/- 1.65	0.4295
SRL 5	180	2.11 +/- 1.32	0.4357

Reviewer's note: ***The GFR was significantly better for placebo vs sirolimus in study 302. However, the serum creatinine was not significantly different in the treatment arms in study 302 at 12 months.***

F. Hemolytic Uremic Syndrome/ Thrombotic Thrombocytopenic Purpura (HUS/TTP)

There were 43 cases of HUS/TTP in studies 301 and 302.

Reviewer's note: ***The observed rates of these events appear to be within the range of that reported in other clinical studies with cyclosporine.***
Note that the rates of HUS are higher for SRL 5 mg.
No patient deaths occurred due to HUS and 3 patients (SRL 5 =2, SRL 2 = 1) lost their grafts.

Rate(%) of HUS/TTP at >12 months

study	SRL 2 mg n=281 study 301 n=218 study 302	SRL 5 mg n=269 study 301 n=208 study 302	Placebo n=130	AZA n=161
301	1.4	2.6	-----	**1.9**
302	2.7	**8.2***	3.2	-------
p-value*	<0.05 SRL 5 vs SRL 2			

G. Hematologic

Please refer to the Applicant briefing package for further discussion of this topic.
Important points:
1)Thrombocytopenia was reported as a dose-related reversible decrease in platelet count and was significantly higher in SRL 5 compared to SRL 2 and AZA and placebo. The applicant states that there were no platelet counts under 50 x 10^9/L after month 3.. Severe thrombocytopenia was rare (0.2%) and although epistaxis is reported in this trial there was only one episodes of epistaxis associated with thrombocytopenia.

2) Leukopenia was significantly more frequent with sirolimus at 5 mg compared to sirolimus at 2 mg per day, but was lower than with azathioprine. There were no cases of neutropenia.(absolute neutrophil count less than 500 per microliter). Leukopenia

resolved with discontinuation of study medication . No white blood cell count was less than 1x 10^9/L (1000 mm^3).

Reviewer's note: ***Leukopenia did not appear to be associated with an increased risk of infection in the sirolimus treatment groups.***

V. SUMMARY OF MAJOR SAFETY ISSUES

1) **Hyperlipidemia is a major issue and will need to be closely followed. It is difficult to ascertain exactly what proportion of patients can be successfully treated with diet and exercise vs lipid-lowering therapy. It is difficult to make any specific recommendations regarding management since treatment decisions will depend on LDL values, which were not available in these studies, and on risk factor stratification/modification.**

2) **The elevated GFR and serum creatinine at the end of 12 months in the sirolimus groups is of concern. The Applicant ascribes this to cyclosporine toxicity however, there was no evidence of elevated cycosporine troughs in this population.**

3) **It would be inappropriate to conclude that 166 black patients encountered less difficulty with infectious disease complications in this study and had decreased efficacy with SRL 2 mg/day because they are "under-immunosuppressed". Factors that predispose immunosuppressed transplant patients to infection are multiple and encompass more than just the type of immunosuppressive agent that they are receiving. To suggest that "more sirolimus" is better for this subset of patients or to recommend the use of the sirolimus 5 mg/day dose must be considered in light of efficacy differences in previously shown tables and be weighed against the potential consequences of hyperlipidemia and vascular disease.**

4) **Although enrollment of African-American patients in Study 301 was excellent, the overall number may be too small to exclude an unacceptable increase in less common adverse events associated with a 5 mg maintenance dose of sirolimus over the long term.**